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Chemical Defenses, Protein Content, and Susceptibility to Herbivory of Diploid vs. Haploid Stages of the Isomorphic Brown Alga *Dictyota ciliolata* (Phaeophyta)

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Seaweeds with free-living diploid and haploid stages might express recessive traits during haploid stages, or exhibit other differences, that would allow those stages to differ in fitness under different environmental conditions. Heteromorphic seaweeds are well known to have variable ecological traits associated with their morphological differences, but ecological differences among isomorphic stages have rarely been investigated. The chemically defended brown alga *Dictyota ciliolata* has a life history with isomorphic alternation of generations, allowing us to assess how chemical traits and susceptibility to herbivory differ among stages. Herbivorous amphipods and sea urchins consumed similar amounts of diploid sporophytes and haploid female and male gametophytes. Concomitant with similar palatability, the different life stages had similar concentrations of soluble protein and levels of chemical defenses. Thus, in addition to morphological similarities, the life stages of *D. ciliolata* appear to share these ecological similarities.

Introduction

Many seaweeds have complex live histories with alternating haploid and diploid stages that are free living (reviewed by John 1994). The morphology of these different phases can be identical (isomorphic) or so strikingly different (heteromorphic) that gametophytic and sporophytic stages have been initially classified in separate families (Dixon 1973). The evolution and adaptive significance of isomorphic versus heteromorphic life histories in seaweeds and the importance of ploidy in otherwise similar life stages is uncertain (Clayton 1988). Seaweeds with life stages that differ in ploidy level might express recessive traits during haploid stages, or express other differences, that would allow stages to vary in fitness under different environmental conditions (Lubchenco and Cubit 1980, Hay and Steinberg 1992). Heteromorphic seaweeds are well known to have their morphological differences associated with variable ecological traits (Lubchenco and Cubit 1980, Slocum 1980, Lewis *et al.* 1987). Ecological differences among isomorphic stages have rarely been investigated. However, isomorphic sporophytes and gametophytes can differ in distribution, suggesting that some ecological specialization may occur among the isomorphic stages (Dixon 1973).

The stages of heteromorphic seaweeds often differ considerably in their physiological and ecological properties. For example, sporophytes and gametophytes of heteromorphic species may differ in productivity, growth, and resistance to grazers (Lubchenco and Cubit 1980, Slocum 1980, Littler and Littler 1983, Clayton 1988, Zupan and West 1990). These differences are hypothesized to allow seaweeds to enhance their performance in habitats where ecological conditions vary significantly in space or time. As an example (Lubchenco and Cubit 1980), upright bladed stages have high growth rates but are easily grazed by herbivores. In contrast, prostrate encrusting forms are more resistant to grazers but have lower growth rates, and are often inferior competitors when herbivores are rare (Lubchenco and Cubit 1980, Slocum 1980, Dethier 1981, Littler and Littler 1983, Lewis *et al.* 1987).

The ecological significance of isomorphic life history patterns are less well studied and less obvious than for heteromorphic species. Some studies have found little, or no, difference between isomorphic stages in photosynthesis, caloric and organic content, or susceptibility to herbivores (Hannach and Santelices 1985, Littler *et al.* 1987, Briting and Chapman 1993). However, morphological similarity does not dictate physiological or ecological similarity, as geographic and local distributions, growth rates, propensity for tearing, and ability to reattach all have been observed to differ between isomorphic life stages (Dixon 1973, Allender 1977, Hannach and Santelices 1985, Juanes and Puente 1993). Additionally, reproductive plants, or plant portions, may be selectively consumed by small herbivores such as amphipods, isopods, and gastropods

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(Moore 1977, Gaines 1985, Gunnill 1985, Watson and Norton 1985), suggesting that isomorphic stages could differ ecologically.

In the case of chemically defended seaweeds, patterns for isomorphic species could be similar to those for heteromorphic species if one phase invests in defenses at the cost of growth, while the alternate phase invests less in defenses but grows faster and thus competes more successfully with other species. If this occurred, one of the seaweed's stages might be expected to be most common in locations or times when herbivores were most active and less common in other situations. In a previous study of algal seasonality and reproduction conducted in North Carolina, Peckol (1982) noted that the brown alga *Dictyota* was present in both gametophytic and sporophytic stages early in the summer (May–July), but only as sporophytic stages later in the summer (August and September). Because species of *Dictyota* in North Carolina are chemically defended from herbivores (Hay *et al.* 1987, Cronin and Hay 1996b,c) and because herbivory in these habitats appears to increase throughout the summer (Hay 1986, Pfister and Hay 1988), this suggested the possibility that different stages of *Dictyota* could be differentially defended and thus differentially adapted to seasonal patterns in herbivory. We tested this hypothesis by determining if male, female, and sporophytic plants of the isomorphic seaweed *Dictyota ciliolata* Kützing differ in their susceptibility to an herbivorous amphipod and sea urchin, and whether patterns of seaweed nutritive value or chemical defenses correspond to patterns of palatability.

Materials and Methods

Study site and organisms

In coastal North Carolina, hard substrate habitats in the photic zone support a high biomass of seaweeds. Grazing pressure in these habitats is seasonally variable but predictable. During warm seasons, large numbers of omnivorous fishes (mostly *Diplodus holbrooki* Bean and *Lagodon rhomboides* L.) and urchins [*Arbacia punctulata* (Lamarck) Philippi] are present (Hay 1986, Pfister and Hay 1988). During colder seasons, the herbivorous fishes migrate to warmer offshore water and the metabolic rate of sea urchins decreases, resulting in less intense grazing pressure. In habitats and seasons when fishes are not consuming most amphipods, some species of these small mesograzers (especially *Ampithoe longimana* Smith) are important consumers of *Dictyota* (Duffy and Hay 1991, 1994, Cronin and Hay 1996b). Although local fishes can be important herbivores on other seaweeds in these habitats (Hay 1986), they were not used in the current study because they do not consume *Dictyota* (Hay *et al.* 1987, 1988).

The chemically defended brown alga *Dictyota ciliolata* Kützing (Cronin and Hay 1996c) is common on hard substrates in North Carolina from April to December (Schneider and Searles 1991). *Dictyota* is not apparent during cold seasons and how it exists during cold winter months when grazing pressure is low has not been investigated. However, a sympatric congener, *Dictyota menstrualis* Hoyt, overwinters as small germlings that begin to grow in the spring as water temperature increases [as *Dictyota dichotoma* (Hudson) Lamouroux in Richardson 1979].

Collection and assay procedures

Sporophytes and female and male gametophytes of *Dictyota ciliolata* used in these experiments were collected from the rock jetty at Radio Island, North Carolina, USA on 29 September 1992. Gametophytes were identified by the presence of antheridia (males) or oogonia (females). Sporophytes were identified by the presence of unilocular sporangia. To determine if the palatability of *Dictyota ciliolata* varied between life stages or sexes, we measured the mass of twenty individuals of each plant type that was consumed by the amphipod *Ampithoe longimana* and the sea urchin *Arbacia punctulata* in laboratory feeding assays. The wet mass of plant portions was determined by spinning the seaweed in a salad spinner to remove excess seawater, quickly weighing the tissue to the nearest mg, and returning it to seawater to avoid undue desiccation. A portion (103 ± 1.3 mg, mean ± 1 SE) of each plant was offered to one of 60 pairs of amphipods and another portion (306 ± 3 mg) of each plant was offered to one of 60 sea urchins (N = 20 for each plant stage × herbivore combination). Changes in mass not due to herbivores was controlled for by placing an additional portion (300 ± 3.3 mg) from each plant in seawater without herbivores. After 2.5 days, the algae were reweighed and the amount of each tissue type consumed from each plant was calculated with the equation $[(H_0 \times C_f/C_0) - H_f)]$; where H_0 and H_f were the mass of the algal portions exposed to herbivory before and after the assay, and C_0 and C_f were the mass of the controls for autogenic changes before and after the assay. These data were analyzed with a one-factor ANOVA, the factor being life stage (i.e., sporophyte, female, male).

Tissue samples for analyses of protein (N = 6) and secondary metabolites (N = 15) were collected as feeding assays were being set up. Samples for protein analysis were frozen, lyophilized, ground into a fine powder, and stored at −25 °C until analyzed. Soluble protein was measured using the Bradford (1976) analysis as modified by Duffy and Hay (1991) with bovine serum albumin as a standard. Although this method is unsatisfactory for determining absolute quantities of protein, it is generally considered reliable for determining relative protein concentration of similar tissues (Davis 1988). The concentration of chemical defenses (dictyol B acetate, pachydictyol,

and dictyodial) produced by *Dictyota ciliolata* was determined using methods of Cronin *et al.* (1995). About 800 mg of tissue were placed in 6 mL of 2 : 1 dichloromethane : methanol (DCM : MeOH) and stored at −25 °C until it was extracted twice with 6 mL of 2 : 1 DCM : MeOH, and analyzed with high performance liquid chromatography (HPLC).

Results and Discussion

We could not detect significant differences in any of the measured plant traits among the isomorphic life stages of *Dictyota ciliolata*. The amphipod *Ampithoe longimana* and the sea urchin *Arbacia punctulata* consumed similar amounts of sporophytes and female and male gametophytes (Fig. 1A and 1B). Plant traits such as protein and secondary metabolites that can be important proximal cues in the feeding behavior of these, and other, herbivores (Hay *et al.* 1987, 1988, Renaud *et al.* 1990, Cronin and Hay 1996a, b) also did not differ significantly among life stages (Fig. 1C, D). However, a non-significant trend (P = .0514, Fig. 1D) suggested that sporophytes might have elevated levels of dictyodial compared to gametophytes. Because dictyodial is relatively unstable (Cronin *et al.* 1995), information of its effects on herbivore feeding is less complete than information for dictyol B acetate and pachydictyol A (Hay *et al.* 1987, Cronin and Hay 1996c). However, available data suggest that dictyodial does not influence the feeding of *Ampithoe longimana*, but that it may deter *Arbacia punctulata* at concentrations found in *Dictyota ciliolata* (Cronin and Hay 1996c).

There was also a weak trend (P = .101) suggesting that protein content of male gametophytes could have been lower than that of sporophytes or female gametophytes (Fig. 1C). The low sample size for this measurement (N = 6) compromises our confidence in this trend. However, the herbivores did not distinguish among any of these life stages, suggesting that any differences in protein content or other plant traits were not great enough to affect the feeding choices.

Previous studies demonstrate that forms of heteromorphic seaweeds often differ markedly in their susceptibility to herbivores (Lubchenco and Cubit 1980, Slocum 1980, Dethier 1981, Lewis *et al.* 1987). Although stages of the isomorphic alga we investigated can differ in their seasonal occurrence (Peckol 1982), and thus exposure to different levels of herbivory, in late summer these stages were ecologically similar in their susceptibility to local herbivores and in their concentrations of protein and defensive metabolites. Littler *et al.* (1987) also found ecological similarities among the isomorphic stages of the red alga *Polycavernosa debilis* (Forsskal) Fredericq *et* Norris: susceptibility to herbivorous reef fishes, photosynthetic rates, and energy content were similar among sporophytes and male and female gametophytes. More seaweeds need to be assayed before a sound conclusion can be reached on general differences, if any, between isomorphic and heteromorphic seaweeds. However, the few studies presently available suggest that the different stages of isomorphic seaweeds are more ecologically similar in their interactions with herbivores

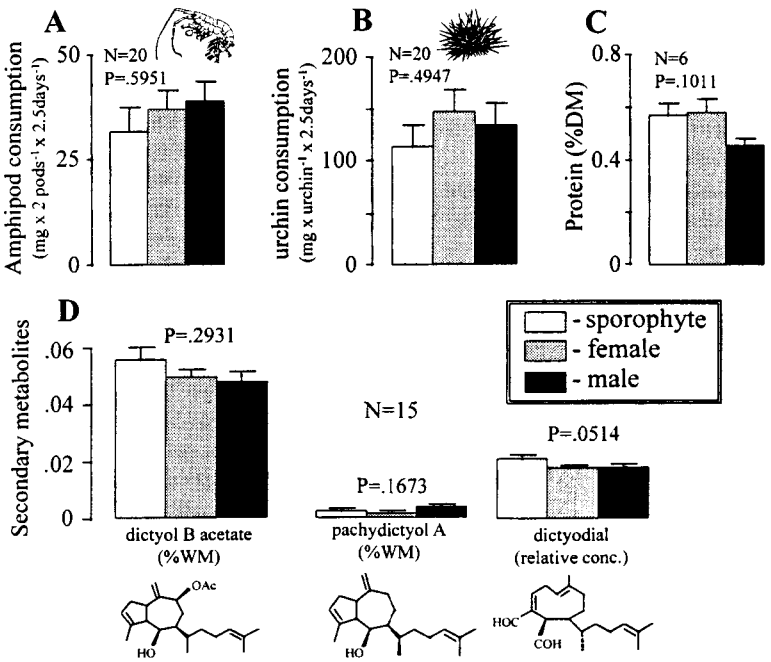

Fig. 1. Comparison of plant traits for the isomorphic life stages of *Dictyota ciliolata*, including the palatability of plants to (A) the amphipod *Ampithoe longimana* and (B) the sea urchin *Arbacia punctulata*, (C) the relative amounts of soluble protein, and (D) the concentrations of secondary metabolites. Bars and error bars represent means + 1 S. E. P-values are from one-factor ANOVAs. The chemical structures of the secondary metabolites are shown below their name.

than the different stages of heteromorphic seaweeds. Thus, differences or similarities of morphological features between life stages of diplohaplontic seaweeds may be useful in predicting differences or similarities in the relationship of the life stages with herbivores.

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